Epigenase™ PRMT Methyltransferase (Type II-Specific) Activity/Inhibition Assay Kit (Colorimetric)

Base Catalog # P-3088

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The Epigenase™ PRMT Methyltransferase (Type II-Specific) Activity/Inhibition Assay Kit (Colorimetric) is suitable for measuring activity or inhibition of total type II PRMT using nuclear extracts or purified enzymes such as PRMT5 and PRMT7 from a broad range of species such as mammals, plants, fungi, and bacteria, in a variety of forms including cultured cells and fresh tissues. Nuclear extracts can be prepared by using your own successful method. For your convenience and the best results, Epigentek offers a nuclear extraction kit (Cat. # OP-0002) optimized for use with this kit. Nuclear extracts can be used immediately or stored at –80°C for future use. Purified enzymes can be active PRMT5 or PRMT7 from recombinant proteins or isolated from cell/tissues.

Input Material: Input materials can be nuclear extracts or purified PRMT5/PRMT7 enzymes. The amount of nuclear extracts for each assay can be 1 µg to 20 µg with an optimal range of 5 to 10 µg. The amount of purified enzymes can be 10 ng to 500 ng, depending on the purity and catalytic activity of the enzymes.

Internal Control: The PRMT assay standard (methylated histone H4-Arg 3) is provided in this kit for the quantification of type II PRMT enzyme activity. Because PRMT activity can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	48 Assays Cat. #P-3088-48	96 Assays Cat. #P-3088-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
PA1 (PRMT Assay Buffer)	4 ml	8 ml	4°C
PA2 (PRMT Substrate, 50X)*	60 µl	120 µl	–20°C
PA3 (Adomet, 50X)*	60 µl	120 µl	–20°C
PA4 (PRMT Assay Standard, 50 µg/ml)*	10 µl	20 µl	–20°C
PA5 (Capture Antibody, 1000X)*	5 µl	10 µl	4°C
PA6 (Detection Antibody, 2000X)*	6 µl	12 µl	–20°C
DS (Developer Solution)	5 ml	10 ml	4°C
SS (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C
Adhesive Covering Film	1	1	RT
User Guide	1	1	RT

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature, and the second and third parts on frozen ice packs at 4°C. Upon receipt: (1) Store **PA2**, **PA3**, **PA4** and **PA6** at –20°C away from light; (2) Store **WB**, **PA1**, **PA5**, **DS**, and **8-Well Assay Strips** at 4°C away from light; and (3) Store remaining components (**SS** and **Adhesive Covering Film**) at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

*Note: (1) Check if **WB** (10X Wash Buffer) contains salt precipitates before use. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; and (2) check if a blue color present in **DS** (Developer Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation

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- ☐ Distilled water
- ☐ Nuclear extract or purified enzymes
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the The Epigenase™ PRMT Methyltransferase (Type II-Specific) Activity/Inhibition Assay Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The Epigenase™ PRMT Methyltransferase (Type II-Specific) Activity/Inhibition Assay Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The Epigenase™ PRMT Methyltransferase (Type II-specific) Activity/Inhibition Assay Kit (Colorimetric) and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

Arginine histone methylation is one of the many important epigenetic marks, and is essential for the regulation of multiple cellular processes. Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs). There are 9 types of PRMTs found in humans but only 7 members are reported to methylate histones. They can mediate mono or dimethylation of arginine residues. These enzymes use S-adenosyl-methionine (SAM) as a methyl donor and transfer it to the guanidinium side chain of arginine. Based on the position of methyl group addition, the PRMTs can be classified into type I (CARM1, PRMT1, PRMT2, PRMT3, PRMT6, and PRMT8) and type II (PRMT5 and PRMT7).

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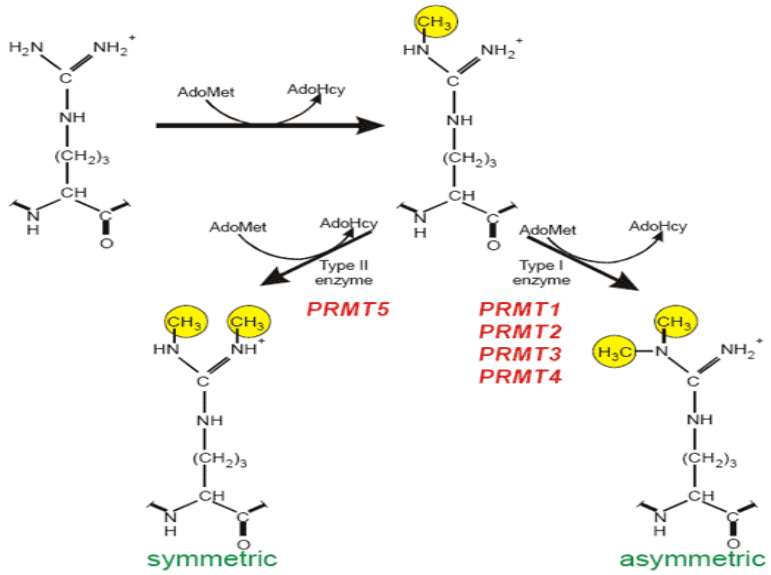

Fig. 1. Histone arginine methylation reaction catalyzed by PRMTs.

Type II PRMTs are found to be strongly implicated in diseases like cancer. For example, PRMT5 plays a role in the repression of certain tumor suppressor genes such as RB tumor suppressors while PRMT7 overexpression is observed in breast cancer. Detection of activity and inhibition of type II PRMTs would be important in elucidating mechanisms of epigenetic regulation of gene activation and silencing, as well as benefiting cancer diagnostics and therapeutics.

The Epigenase™ PRMT Methyltransferase (Type II-Specific) Activity/Inhibition Assay Kit (Colorimetric) is designed to detect type II PRMTs including PRMT5 and PRMT7 activity and inhibition. This kit has the following advantages:

- 3 hour colorimetric procedure in a 96 stripwell microplate format allows for either manual or high throughput analysis.
- Directly measures type II PRMT activity via a straightforward detection of PRMT-converted methylated products.
- Both cell/tissue extracts and purified type II PRMT can be used, which allows for the detection of inhibitory effects of PRMT5 or PRMT7 inhibitors *in vivo* and *in vitro*.
- Sensitive detection limit can be as low as 5 ng of purified PRMT5 enzyme.
- Methylated H4-Arg3 standard is included, allowing for specific activity of type II PRMT to be quantified.
- Accurate, reliable, and consistent with extremely low background signals.

PRINCIPLE & PROCEDURE

In this assay, a type II PRMT substrate is stably coated onto microplate wells. Active PRMT5 or PRMT7 bind to the substrate and transfer a methyl group from Adomet to methylate the substrate. The methylated products can be recognized with a specific antibody. The ratio or amount of methylated products, which is proportional to enzyme activity, can then be colorimetrically measured by reading the absorbance in a microplate spectrophometer at a wavelength of 450 nm. The activity of the type II PRMT enzymes is in turn proportional to the optical density intensity measured.

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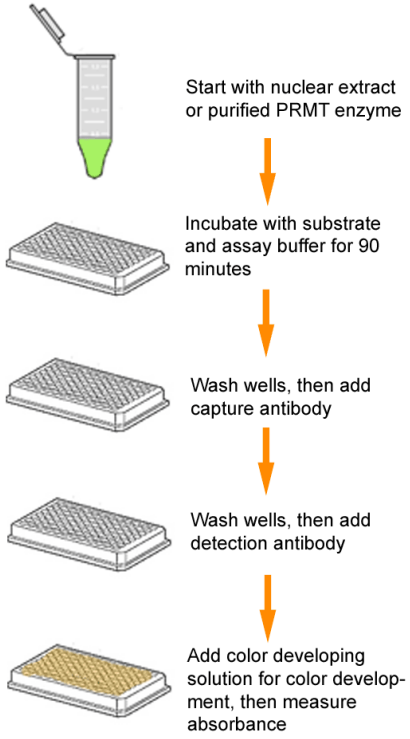
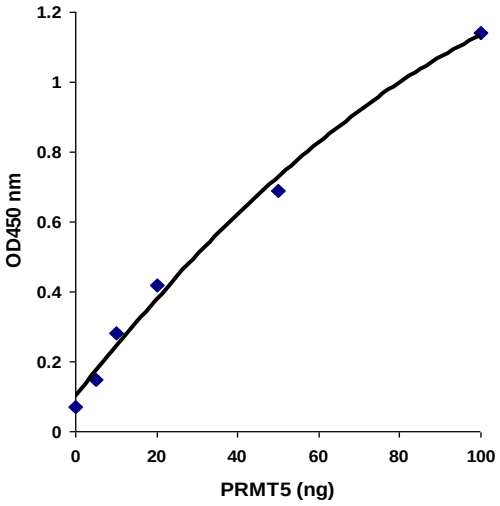
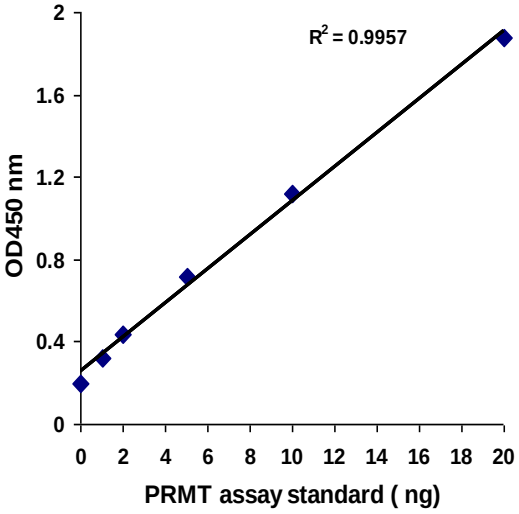

Start with nuclear extract or purified PRMT enzyme
Incubate with substrate and assay buffer for 90 minutes
Wash wells, then add capture antibody
Wash wells, then add detection antibody
Add color developing solution for color development, then measure absorbance

Schematic procedure of the Epigenase™ PRMT Methyltransferase (Type II-Specific) Activity/Inhibition Assay Kit (Colorimetric)



Demonstration of high sensitivity of the type II PRMT activity assay achieved by using recombinant PRMT5 with the Epigenase™ PRMT (Type II-Specific) Methyltransferase Activity/Inhibition Assay Kit (Colorimetric).



Illustrated standard curve generated with type II PRMT assay standard.

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PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of nuclear extracts for each assay can be between 1 µg and 20 ug with an optimal range of 5 to 10 µg. The amount of purified enzymes can be between 10 ng and 500 ng, depending on the purity and catalytic activity of the enzymes.

Nuclear Extraction: You can use your method of choice for preparing nuclear extracts. Epigentek also offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit.

Nuclear extracts or PRMT enzyme should be stored in aliquots at –80°C until use.

1. Working Buffer and Solution Preparation

a. Prepare **Diluted WB** 1X Wash Buffer:

48-Assay Kit: Add 13 ml of **WB** 10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **Diluted PA5** Capture Antibody Solution:

Dilute **PA5** Capture Antibody with **Diluted WB** 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µl of capture antibody to 1000 µl of 1X Wash Buffer). 50 µl of **Diluted PA5** will be required for each assay well.

c. Prepare **Diluted PA6** Detection Antibody Solution:

Dilute **PA6** Detection Antibody with **Diluted WB** 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µl of **PA6** Detection Antibody to 2000 µl of **Diluted WB** 1X Wash Buffer). 50 µl of **Diluted PA6** will be required for each assay well.

d. Prepare **Diluted PA4** Standard Solution:

Suggested Standard Curve Preparation: First, dilute **PA4** Assay Standard with **PA1** Assay Buffer to 20 ng/µl by adding 4 µl of **PA4** to 6 µl of **PA1**. Then, further prepare five concentrations by combining the 20 ng/µl **Diluted PA4** with **PA1** into final concentrations of 1, 2, 4, 10, and 20 ng/µl according to the following dilution chart:

Tube	PA4 (20 ng/µl)	PA1	Resulting PA4 Concentration
1	1.0 µl	19.0 µl	1 ng/µl
2	1.0 µl	9.0 µl	2 ng/µl
3	1.0 µl	4.0 µl	4 ng/µl
4	2.0 µl	2.0 µl	10 ng/µl
5	4.0 µl	0.0 µl	20 ng/µl

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*Note: Keep each of the diluted solutions except **WB** 1X Wash Buffer on ice until use. Any remaining diluted solutions other than **Diluted WB** should be discarded if not used within the same day.*

2. Enzymatic Reaction

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Blank Wells: Add 49 µl of **PA1** and 1 µl of **PA3** to each blank well.

c. Standard Wells: Add 49 µl of **PA1** and 1 µl of **Diluted PA4** to each standard well with a minimum of five wells, each at a different concentration between 1 and 20 ng/µl (based on the dilution chart in Step 1e; see Table 3 as an example).

d. Sample Wells Without Inhibitor: Add 44 to 47 µl of **PA1**, 1 µl of **PA2,**1 µl of **PA3**, 1 to 4 µl of your nuclear extract or 1 to 4 µl of purified PRMT enzyme. Total volume should be 50 µl per well.

e. Sample Wells With Inhibitor: Add 39 to 42 µl of **PA1**, 1 µl of **PA2,** 1 µl of **PA3**, 1 to 4 µl of your nuclear extract or 1 to 4 µl of purified PRMT enzyme, and 5 µl of inhibitor solution. Total volume should be 50 µl/well.

*Note: (1) Follow the suggested well setup diagrams; (2) It is recommended to use 2 µg to 10 µg of nuclear extract per well or 20 ng to 100 ng of purified enzyme per well; (3) The concentration of inhibitor to be added into the sample wells can be varied (e.g., 1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with **PA1** at a 1:10 ratio (e.g., add 0.5 µl of inhibitor to 4.5 µl of **PA1**), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.*

Tightly cover strip-well microplate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60 to 120 min.

*Note: (1) The incubation time may depend on intrinsic JMJD2 activity. However, in general, 60-90 min incubation is suitable for active purified PRMT enzymes and 90-120 min incubation is required for nuclear extracts; (2) The **Adhesive Covering Film** can be cut to the required size to cover the strips based on the number of strips to be used.*

f. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

3. Antibody Binding & Signal Enhancing

a. Add 50 µl of the **Diluted PA5** to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.

b. Remove the **Diluted PA5** solution from each well.

c. Wash each well three times with 150 µl of the **Diluted WB** each time.

d. Add 50 µl of the **Diluted PA6** to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min.

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e. Remove the **Diluted PA6** solution from each well.

f. Wash each well four times with 150 µl of the **Diluted WB** each time.

Note: Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.

4. Signal Detection

a. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient methylated products.

b. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: (1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

5. PRMT Activity Calculation

a. Calculate the average duplicate readings for the sample wells and blank wells.

b. Calculate PRMT activity or inhibition using the following formulas:

For simple calculation:

$$PRMT\ Activity\ (OD/min/mg) = \frac{(Sample\ OD - Blank\ OD)}{(Protein\ Amount\ (\mu g)^{*} \times min^{**})} \times 1000$$

* Protein amount (µg) added into the reaction at step 2d.
** Incubation time (minutes) at step 2f.

Example calculation:

Average OD450 of sample is 0.65
Average OD450 of blank is 0.05
Protein amount is 5 µg
Incubation time is 120 minutes (2 hours)

$$\text{PRMT activity} = \frac{(0.65 - 0.05)}{(5 \times 120)} \times 1000 = 1\ \text{OD/min/mg}$$

For accurate or specific activity calculation:

1. Generate a standard curve and plot OD value versus amount of **PA4** at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of PRMT-converted demethylated product using the following formulas:

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$$Methylated\ product\ (ng) = \frac{(Sample\ OD - Blank\ OD)}{Slope}$$

$$PRMT\ Activity\ (ng/min/mg) = \frac{Methylated\ Product\ (ng)}{(Protein\ Amount\ (\mu g) \times min^*)} \times 1000$$

* Incubation time (minutes) at Step 2f.

For inhibition calculation:

$$Inhibition\ \% = \left[1 - \frac{Inhibitor\ Sample\ OD - Blank\ OD}{No\ Inhibitor\ Sample\ OD - Blank\ OD} \right] \times 100\%$$

SUGGESTED BUFFER AND SOLUTION SETUP

Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
PA1	50 µl	400 µl	800 µl	2400 µl	4800 µl
PA2	1 µl	8 µl	16 µl	50 µl	120 µl
PA3	1 µl	8 µl	16 µl	50 µl	120 µl
PA4	N/A	N/A	4 µL (optional)	8 µl	8 µl
Diluted PA5	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted PA6	50 µl	400 µl	800 µl	2400 µl	4800 µl
Developer Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
Stop Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

SUGGESTED STRIP WELL SETUP

Table 2. The suggested strip-well plate setup for PRMT activity assay in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	PA4 1 ng	PA4 1 ng	Sample	Sample	Sample	Sample
C	PA4 2 ng	PA4 2 ng	Sample	Sample	Sample	Sample
D	PA4 4 ng	PA4 4 ng	Sample	Sample	Sample	Sample
E	PA4 10 ng	PA4 10 ng	Sample	Sample	Sample	Sample
F	PA4 20 ng	PA4 20 ng	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

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TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	The well is incorrectly washed before enzyme reaction.	Ensure the well is not washed prior to adding the positive control and sample.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 2c.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of **PA4** (PRMT Assay Standard).
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with **Diluted PA6** is too long.	The incubation time at Step 3d should not exceed 2 hours.
	Over-development of color.	Decrease the development time in Step 4a before adding **SS** Stop Solution in Step 4b.
No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified.	Ensure your protocol is suitable for PRMT protein extraction. For the best results, it is advised to use Epigentek's Nuclear Extraction Kit (Cat. No. OP-0002). Also, use fresh cells or tissues for protein extraction,

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		as frozen cells or tissues could lose enzyme activity.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of purified enzymes or nuclear extracts is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure sample is stored in aliquots at –80°C, with no more than 6 weeks for nuclear extracts and 6 months for purified enzymes. Avoid repeated freezing/thawing.
	Little or no activity of PRMT contained in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared nuclear extracts or purified enzymes.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12).

RELATED PRODUCTS

Nuclear Extract Preparation

OP-0002-1 EpiQuik™ Nuclear Extraction Kit

Histone Demethylase Activity/Inhibition Assay

P-3077 EpiQuik™ Histone Demethylase (H3-K9 Specific) Activity/Inhibition Fast Assay Kit
P-3078 Epigenase™ LSD1 Demethylase Activity/Inhibition Assay Kit (Colorimetric)
P-3079 Epigenase™ LSD1 Demethylase Activity/Inhibition Assay Kit (Fluorometric)
P-3081 Epigenase™ JMJD2 Demethylase Activity/Inhibition Assay Kit (Fluorometric)
P-3082 Epigenase™ JARID Demethylase Activity/Inhibition Assay Kit (Colorimetric)
P-3083 Epigenase™ JARID Demethylase Activity/Inhibition Assay Kit (Fluorometric)

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